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The influence of human interaction on guinea pigs: behavioral and thermographic changes during animal-assisted therapy

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Abstract

Effects of human contact, retreat and presence of conspecifics on stress and wellbeing of guinea pigs involved in animal-assisted therapy (AAT) were investigated. Twenty animals were tested in four settings in a randomized, controlled within-subject trial with repeated measurements: (I) therapy with retreat possibility with conspecifics, (II) therapy with retreat possibility without conspecifics, (III) therapy without retreat possibility without conspecifics, (IV) control without any human interaction. The eye temperature was measured with video thermography. Behaviors (individual behavior, social behavior, human-animal-interaction, interaction with environment) were coded with an ethogram.

Temperature was rising in settings I and III. Animals were spending less time eating in settings I – III than in the control (IV). With retreat (I) they showed more active behaviors (locomotion, startling) whereas without retreat (III) they showed more passive behaviors (standing still, freezing).

Reduced eating, increased starling and freezing were identified as stress indicators. Key factor for stress might be petting. We conclude that guinea pigs involved in AAT should have retreat possibility, especially when petted, have access to conspecifics and time to adapt to a new setting. In this way, stress can be reduced, and AAT might potentially even constitute enrichment for the involved animals.

Keywords: animal-assisted therapy, guinea pig, behavior, stress, thermography, human-animal interaction

Zusammenfassung

Es wurden Effekte von menschlicher Interaktion, Rückzugsmöglichkeit, sowie der Anwesenheit von Artgenossen auf Stress und Wohlbefinden bei Meerschweinchen in tiergestützten Therapien untersucht. In einer randomisierten, kontrollierten within-subject Studie mit wiederholten Messungen wurden vier Settings getestet: (I) Therapie mit Rückzug, (II) Therapie ohne Artgenossen, (III) Therapie ohne Rückzug, (IV) Kontrolle ohne menschliche Interaktion. Videothermographisch wurde die Augentemperatur gemessen. Das Verhalten (individuelles Verhalten, Sozialverhalten, Mensch-Tier-Interaktionen, Umweltinteraktionen) wurde anhand eines Ethogramms kodiert.

In Settings I und III stieg die Temperatur an. In Settings I – III wurde kürzer gefressen als in der Kontrolle (IV). Mit Rückzug (I) wurde es mehr aktives Verhalten (Bewegung, Aufschrecken) gezeigt. Ohne Rückzug (III) gab es mehr passives Verhalten (Stillstehen, Schockstarre).

Reduziertes Fressen, vermehrtes Aufschrecken und Schockstarre wurden als Stress-Indikatoren identifiziert. Schlüsselfaktor für Stress könnte das Streicheln sein. Wir schlussfolgern, dass Meerschweinchen, wenn sie in tiergestützten Therapien involviert werden, eine Rückzugsmöglichkeit haben müssen, vor allem dann, wenn sie gestreichelt werden. Sie müssen Zugang zu Artgenossen haben und Zeit erhalten, sich an neue Situationen zu adaptieren. So kann Stress reduziert werden und tiergestützte Therapien können möglicherweise sogar bereichernd für die involvierten Tiere sein.

Schlüsselwörter: tiergestützte Therapie, Meerschweinchen, Verhalten, Stress, Thermographie, Mensch-Tier Interaktion

The influence of human interaction on guinea pigs: behavioral and thermographic changes during animal assisted therapy

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Keywords: animal-assisted therapy, guinea pig, behavior, stress, thermography, human-animal interaction

Highlights

(1) Responses of guinea pigs to animal-assisted therapy were investigated.
(2) Eye temperature and behavior were measured in different settings.
(3) Human contact, presence of a retreat and conspecifics influence guinea pigs' welfare.
(4) Petting might lead to stress in guinea pigs.
(5) Reduced eating, increased startling and freezing might be stress indicators.

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Abstract

Guinea pigs are often involved in animal-assisted therapy (AAT) but there is little knowledge about the effects of human contact on guinea pigs involved in AAT. The aim of this study was to investigate effects of human contact, availability of a retreat and presence of conspecifics on indicators of welfare in guinea pigs involved in AAT. Guinea pigs of both sexes and different ages (n=20) were assigned to a randomized, controlled within-subject trial with repeated measurements. Each guinea pig was tested in four settings: (I) therapy with retreat possibility with conspecifics, (II) therapy with retreat possibility without conspecifics, (III) therapy without retreat possibility, and (IV) control without any human interaction. We measured changes in eye temperature, as a proxy to infer stress levels, at 5-s intervals with a thermographic camera. All sessions were video recorded and the guinea pigs' behavior was coded using continuous recording and focal animal sampling. For the statistical analysis we used generalized linear mixed models, with therapy setting as a fixed effect and individual guinea pig as a random effect.

We observed a temperature increase relative to baseline in settings (I) therapy with retreat with conspecifics present and (III) therapy without retreat. The percentage of time a guinea pig was petted was positively correlated with a rise in the eye temperature independent of the setting. Time spent eating was reduced in all therapy settings (I-III) compared to the control (IV). In the setting with retreat (I), guinea pigs showed more active behaviors such as locomotive behavior or startling compared to the setting without retreat (III) and the control setting (IV). When no retreat was available (III), they showed more passive behaviors, such as standing still or freezing compared to therapy with retreat (I).

Based on our results we identified the behaviors "reduced eating", "increased startle" and "increased freezing" as indicators of an increased stress level. Petting the guinea pigs was correlated with a rise in the eye temperature and could be a factor which causes stress in guinea

pigs. We conclude that guinea pigs involved in AAT should have a retreat possibility, especially when petted, should have access to conspecifics, and should be given time to adapt to a new setting. In this way, stress can be reduced, and AAT might potentially even constitute enrichment for the involved animals.

1. Introduction

Guinea pigs are part of various animal-assisted interventions, but there is a lack of knowledge regarding the effects of their involvement in such interventions or of human contact in general on their welfare, comprising physical and emotional state [1,2]. Animal-assisted therapy (AAT) is a form of animal-assisted interventions where an animal is involved in a therapeutic setting. This approach is often used for people who are difficult to reach using conventional therapeutic methods. AAT is planned and structured by trained professionals with the goal to improve emotional, social and physiological functioning of the patient [3]. Recent research shows that involving animals in interventions has numerous positive effects on humans' social behavior, emotional states such as anxiety or depression, and physiological parameters such as blood pressure, heart rate or respiratory rate [4,5].

Guinea pigs (*Cavia aperea f. porcellus*) are a common species in animal-assisted interventions. Research shows that interacting with guinea pigs can have positive effects on patients' social behavior [6] and their ability to make contact and communicate [7]. Guinea pigs are social and curious animals [8]. They are easy to keep and handle, which makes them suitable for AAT. However, the guinea pig is a prey species and stress-prone. In order to carry out AAT in an ethical manner and with respect to One Health, it is crucial to have adequate knowledge about behavior, needs and health as well as indicators and methods for regulation of stress of the involved animals [3]. It is an ethical obligation to scientifically examine the effects on the animals involved in AAT to avoid a tradeoff of human against animal wellbeing [9] . One Health recognizes the inseparable linkage between humans, animals and their environment and is defined as added value in human and animal health and wellbeing that is achieved by a closer cooperation of different disciplines [9–11]. To understand this link within AAT, research about effects on the involved animals is crucial. Knowledge of how negative effects on the animals

can be reduced is needed as well as knowledge on how conditions should be designed so that both the patient and the animals can benefit.

Effects of AAT on the involved animals are increasingly scientifically investigated especially in dogs [12,13] and horses [14], with mixed results. However, only two studies have investigated guinea pigs in AAT so far [15,16]. Gut and colleagues [15] investigated the behavior of five female guinea pigs during AAT with and without retreat possibility, in comparison to a control setting with retreat possibility and no human interaction. The study provided evidence that the possibility to retreat is a key factor to reduce stress in guinea pigs and should always be provided during AAT. The limiting factors of the study were the small sample size and that it remained unclear how the behavioral observations were related to physiological reactions of the guinea pigs. Therefore, we wanted to combine behavior with physiological data in the present study.

Physiological stress in guinea pigs has been studied noninvasively using saliva cortisol levels [17] or fecal glucocorticoid metabolite concentrations [18]. However, these methods have limitations, as described in previous studies on other animal species, e.g. regarding the correlation with blood cortisol levels [19]. To avoid these problems, we measured physiological stress via infrared thermography. Infrared thermography is a relatively new method of non-invasive stress quantification which measures body temperature from a distance [20]. Body temperature is a very sensitive stress parameter and allows for real-time information about physiological stress processes. Stress is associated with different autonomous, endocrinal and neurochemical changes as well as behavioral changes [1,21]. These prepare an organism for potential threats [22] leading to a rise of the internal body temperature (hyperthermia) within a short time [22] and a decrease of the outer body temperature in the extremities due to vasoconstriction [23]. A thermal imaging camera features infrared sensors which make radiometric measurements of the outside body temperature. This technology is very precise concerning temperature measurement and spatial resolution [24,25]. Numerous studies have

shown that eye temperature of various mammalian species rises in stressful situations and is correlated with core body temperature (e.g. cattle [26], horses [27], dogs [28]). A study with chickens indicated that even the intensity of the stressor can be evaluated with a thermographic camera [23]. Furthermore, a thermographic video camera allows for video recording of animals in the absence of humans [29].

The aim of this study was to investigate the effects of human contact, a retreat possibility and presence of conspecifics on stress and wellbeing in guinea pigs involved in AAT using video thermography and behavioral observations. Based on our previous study [15], we proposed three hypotheses. (1) Provision of a retreat and giving the animal free choice of interactions are associated with reduced physiological and behavioral stress indicators. (2) The presence of conspecifics leads to reduced physiological and behavioral stress indicators during AAT. (3) Human-animal interaction could be an enrichment for the guinea pigs by encouraging them to make contact with humans, interact more with their environment and discover new situations.

2. Materials and Methods

This study was approved by the Veterinary Office of the canton Basel-Stadt, Switzerland (N° of approval: 2713). It was conducted in accordance with the Animals (Scientific Procedures) Act 1986, European Directive EU 2010/63, and the Guidelines for the Use of Animals in Research of the Association for the Study of Animal Behavior and the Animal Behavior Society. AAT was performed according to the IAHAIO guidelines [3]. Break-off criteria were defined as an excessive display of stress-associated behaviour by the animal. No session was stopped early, and no adverse incidents occurred.

2.1 Subjects

Twenty guinea pigs (*Cavia aperea f. porcellus*) of mixed sources, breeds, sexes and different levels of experience with AAT were part of this study (Table 1). They were identifiable by natural markings. Six guinea pigs (five intact females, one castrated male), kept in two groups of three animals each (group 1 and 2), had been involved in AAT at REHAB Basel, a rehabilitation clinic in Switzerland, on a regular basis with up to two therapy sessions per day for at least one year. Fourteen guinea pigs (10 intact females, four castrated males) were provided by private households and had not been involved in AAT before. They were kept in six groups of two to three animals, respectively (groups 3 to 6).

Table 1: Subject characteristics

	Group 1	**Group 2**	**Group 3**	**Group 4**	**Group 5**	**Group 6**	**Group 7**	**Group 8**
Group size	3	3	3	2	2	2	3	2
Gender	3 ♀	2 ♀, 1 ♂	3 ♀	2 ♀	2 ♀	2 ♂	2 ♀, 1 ♂	1 ♀, 1 ♂
Experience with AAT	Yes	Yes	No	No	No	No	No	No
From private households	No	No	Yes	Yes	Yes	Yes	Yes	Yes
Ages (years)	6.5, 7.5, 7.5	1.5, 5.5, 5.5	2, 6, ?	6, ?	2, ?	5, 5	1.5, 3, 3	1.5, 1.5

♀: female, ♂: castrated male, ?: exact age unknown but older than one year

One female guinea pig of group 7 died unexpectedly during the data collection phase without showing clinical signs of illness prior to death. A post-mortem examination was carried out at

the Institute of Veterinary Pathology in Zurich (case N° S19-0788). Sepsis due to *Streptococcus pneumoniae* was identified as the cause of death. The data on this animal were not included in the analysis. Data collection stopped immediately after the incident due to implementation of hygiene measures which led to some missing data from two other guinea pigs. This led to a final sample size of 19 investigated guinea pigs.

2.2 Handling and housing

During the study, each group of guinea pigs was housed in a two-story cage (Figure 1) of 3 m^2 in accordance with Swiss standards for animal welfare. The cage contained shelters, twigs, wood shavings, hay, straw and a bowl of water. The guinea pigs had access to hay, straw and water ad libitum. We fed them three times a day with fresh vegetables, herbs and grains. For guinea pigs from private households, we collected the information about handling, housing and feeding prior to the study start using a questionnaire filled in by the animal owners.

Figure 1: Housing of the guinea pig groups in a two-story cage of 3 m^2

All animals were housed and handled under similar conditions, whether originating from private households or residing at the rehabilitation center. External animals arrived two weeks before the study start to acclimatize in the two-story cages prior to data collection. During this time, the guinea pigs acclimatized in their cages and were not handled otherwise. In the second week, they were transferred to and hand-fed in the table enclosure used for therapies (Figure 2) daily to familiarize them with the study setting. During the third week, we collected the data.

2.3 Study design

The study followed a randomized controlled within-subject design with repeated measurements. We observed each guinea pig twice in four different settings.

(I): Therapy setting with retreat and access to conspecifics: animals had free choice of human interaction or retreat into their enclosure.

(II): Therapy setting with retreat without access to conspecifics: one single animal had free choice between human-interaction and retreat in its table enclosure.

(III): Therapy setting without retreat without access to conspecifics: one single animal was placed on a plush pet bed in the lap of the patient.

(IV): Control setting without human interaction with access to conspecifics: animals were in the table enclosure without a patient or another human present.

A total of 147 observations were made, consisting of two observations per guinea pig per setting. Due to the previously described data loss, the final analysis for both physiology and behavior included: (I) Therapy with retreat n = 38, (II) therapy without conspecifics n = 36, (III) therapy without retreat n = 36, (IV) control n = 37.

The experiments were carried out with one test subject rather than actual patients (hereafter referred to as "patient") in order to standardize the interactions as much as possible. Each guinea pig was tested in one to two settings per day in different setting combinations, to simulate real AAT sessions as performed at REHAB Basel, using a crossover design. This resulted in combinations of setting I with II, III or none as well as combinations of setting IV with II, III or none. The order of the settings and their distribution over the different trial days were predetermined in a randomized order. Carry-over effects were accounted for by testing each setting combination on different days and taking breaks of 10 minutes between two settings within a combination. Settings are described below. Settings I, III and IV were set up similar to the pilot study of Gut and colleagues [15].

All four settings took place in a room designated for AAT at REHAB Basel. Each session started with the transfer of the guinea pigs from their cage to the table enclosure followed by habituation of 30 minutes in the table enclosure. During this time, we kept ambient noise and activity to a minimum. Settings I, II and IV took place in a table enclosure specially designed for AAT with guinea pigs. A 1.2 m^2 table framed with a Plexiglas wall was set up in a standardized way with bedding, shelters (two hay tunnels and two wooden houses), hay, straw, twigs and a water bowl (Figure 2), referred to as "enclosure part". Adjacent to the front was an attached wooden front table, hereafter referred to as "board part", on which there was a plush pet bed. Both areas, the enclosure part as well as the board part, were freely accessible to the guinea pigs and offered opportunity to interact with humans or retreat in a shelter. In setting III the guinea pig was placed in the plush pet bed on the lap of the patient.

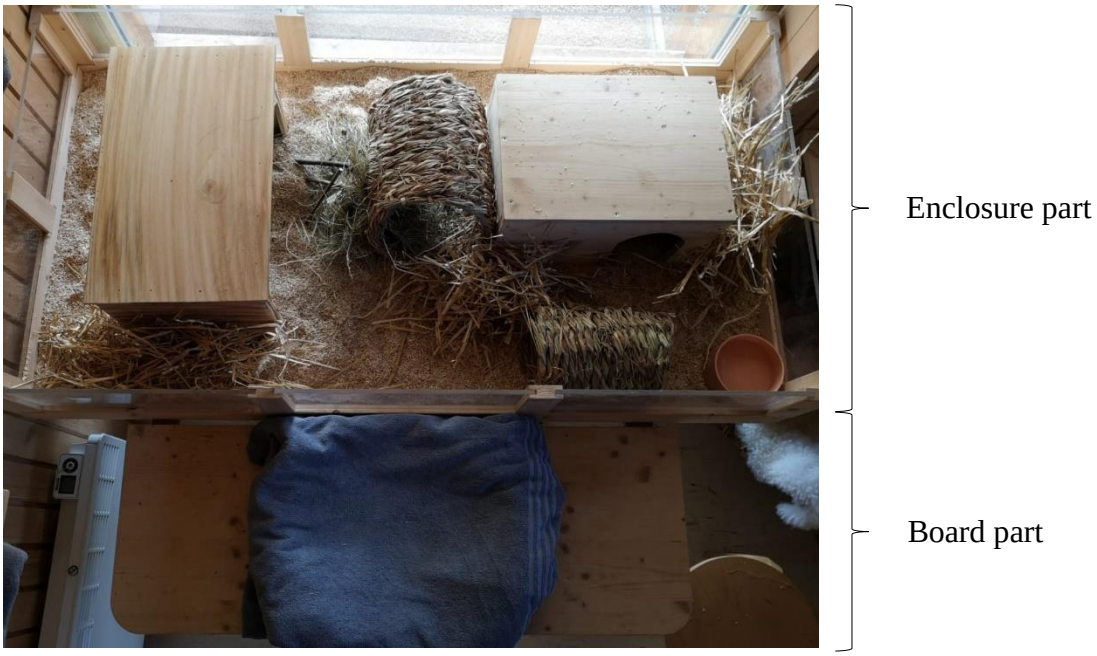

Figure 2 Table enclosure divided in the two areas: Enclosure part and board part.

2.3.1 Setting I: Therapy setting with retreat possibility and access to conspecifics

In the therapy setting with retreat possibility, the grouping of guinea pigs in the table enclosure was always according to their original social group. The setting duration was 30 minutes. The patient tried to encourage the animals to approach with fresh succulent food (60 g of vegetables, i.e. carrots, celery stalks, lettuce, grass and dandelions, per animal). The guinea pigs were encouraged to gather pieces of vegetable from wooden pet puzzle toys, branches with holes or a wooden board with holes. The patient fed and petted the animals on the board part, while they had the possibility to retreat into the shelters at any time during therapy.

2.3.2 Setting II: Therapy setting with retreat possibility without access to conspecifics

In the therapy setting without interaction with conspecifics, the setting was identical to the therapy setting with retreat except that the animal had no contact with their social group. One guinea pig was in the table enclosure for five minutes without its social partners, but had visual, olfactory and auditory contact with its group in the same room. The patient tried to attract the animal with fresh succulent food (20 g of vegetables, e.g. carrots, celery stalks, lettuce, grass

and dandelions per animal). The guinea pig was encouraged to gather vegetable pieces from wooden pet puzzle toys, branches with holes or a wooden board with holes. The patient fed and petted the animal on the board part, while it had the possibility to retreat into shelters at any time during therapy.

2.3.3 Setting III: Therapy setting without retreat possibility without access to conspecifics

In the therapy setting without retreat possibility, one guinea pig was placed in the pet bed on the lap of the patient for five minutes while having visual, olfactory and auditory contact with its social partners in the same room. The patient fed the guinea pig with fresh succulent food (20 g of vegetables e.g. carrots, celery stalks and lettuce, grass and dandelions per animal) and petted the animal. The guinea pig did not have the possibility to leave the lap to retreat into shelter. Break-off criteria were defined as an excessive display of stress-associated behaviour by the animal (e.g. piloerection, eye-closing or attempts to jump out of the pet bed on the patient's lap).

2.3.4 Setting IV: Control setting without human interaction with access to conspecifics

In the control setting, the guinea pigs were in the table enclosure for 30 minutes in their social group. There was no patient present and no human-animal interaction. The guinea pigs had free access to the enclosure part and the board part. They could approach fresh succulent food (60 g of vegetables, e.g. carrots, celery stalks and lettuce, grass and dandelions per animal) distributed on the board part and the front enclosure part, which were the areas that would also be accessible by humans during therapy with retreat (see setting I).

2.4 Data collection

2.4.1 Physiological measurements

Eye temperature was measured using the thermography video camera FLIR T530 with a wide angle (41°) objective. The camera was calibrated for reflecting temperature, consisting of living (e.g. animals, humans) and non-living (e.g. heating, solar irradiation) components in the surroundings of the measured animal before every setting.

Temperature data were collected using continuous video recording at a distance of approx. one meter between the objective and the focal animal at an angle of approx. 90°. The image analysis software FLIR Tools (Version 5.13.18031.2002) was used to measure the maximum temperature (°C) within an oval area traced around the eye, including the eyeball and approx. 0.5 cm around the outside of the eyelids. For analysis, the thermographic videos were divided into five-second intervals and the absolute maximum value for each interval was exported. On this basis, an mean temperature value per condition was calculated. The temperature change relative to the baseline temperature was used for the data analyses. The baseline temperature of each animal was determined by a mean of measured values during the last 15 minutes of the habituation phase in the table enclosure before the start of the therapies/control.

2.4.2 Behavior observations

All sessions were filmed using a video camera (Sony™ Camcorder). For coding, the video recordings were trimmed to contain one setting each. In that way, the video coder was blinded with regard to setting order. It was not possible to blind for the type of setting, because this was visibly obvious to the coder.

Animal behavior was analyzed using continuous recording and focal sampling [30] by coding the videos with Noldus Observer XT 12.5 according to the ethogram designed by Gut and colleagues [15]. All videos were coded by one trained coder. Intra-rater reliability ranged

between 0.84 and 0.99 and inter-rater reliability with the observer of our previous study [15] was between 0.88 and 0.93, as measured by Cohen's kappa [31].

The following behaviors were observed according to our previously developed ethogram (see appendix, Table 5) [15] and served as dependent variables:

a) Individual behavior: ingestive behavior, locomotive behavior, comfort behavior

b) Interactions with the environment: explorative behavior, non-explorative behavior

c) Social behavior towards conspecifics: sociopositive behavior, general socionegative behavior

d) Active human animal interaction (HAI): sociopositive HAI, general socionegative HAI

e) Passive human animal interaction: being petted

f) Vocalisation

g) Other groups: visibility (guinea pig is in view), unexpected behavior (i.e. a sound from outside), observation on-going (start to end of the setting without pre-/postprocessing)

Frequencies of short countable behaviors were calculated as n/60 s. Longer enduring state behaviors were calculated as percentages of the observed time. For most behaviors, the denominator "visible and on-going" was used. This ensured that the reference time (100%) only counted when the therapy was on-going and the animal was visible in the camera. For "vocalization," "hiding," "on board part," and "in enclosure unsheltered," only the denominator "on-going" was used because these behaviors also occurred when the animal was not visible.

2.5 Data processing and statistical analysis

We used IBM SPSS Statistics, Version 23.0, for all analyses and considered *P* values < 0.05 as statistically significant.

2.5.1 Physiological measurements

Thermography data were calculated as the temperature change relative to the baseline temperature in °C. A generalized linear mixed model with setting (I, II, III or IV) as fixed effects and individual guinea pig as random effect were used. As effect size, the linear coefficient (b) was calculated, i.e., the difference in the temperature changes. Influence of additional factors (day of data collection, sex, room temperature, previous AAT experience, percentage of time being petted) was tested by including them as covariates into the model. Model diagnostics included visual checks for normality of residuals. All residuals were approximately normally distributed.

2.5.2 Behavior observations

Data analysis was performed analogously to Gut and colleagues [15].

We analyzed countable behavior data using a generalized linear mixed model with Poisson distribution. In case of overdispersion, observed via deviance (DF), we fitted a model using the negative binomial distribution. Setting was used as a fixed effect, and individual guinea pigs were used as random effect. The logarithmized duration of the session was specified as an offset variable to correct the video length so that each video had the same impact regardless of its length. As effect size, the incidence rate ratio (IRR) was calculated, that is, the relative change in the rates of the observed event. To analyze the data of state behaviors, the arcsine transformed percentage of the analyzed time in total was used. A generalized linear mixed model with setting (I, II, III or IV) as fixed effects and individual guinea pig as random effect was used. As effect size, the linear coefficient (b) was calculated, that is, the difference in the proportions but estimated on arcsine scale. Influence of additional factors (day of data collection, sex, room temperature, previous AAT experience) was tested by including them as covariates into the models. The following behaviors were shown too rarely to be analyzed: Resting, jumping, drinking, sociopositive social behavior, socionegative social behavior, sociopositive HAI.

Model diagnostics included visual checks for normality of residuals. All residuals were approximately normally distributed.

3. Results

3.1 Physiology: Eye temperature compared between different settings

Table 2: Relative changes in eye temperature (difference to baseline) compared between different settings.

Setting	N	M	SD	Coef	95% CI	*P* value	
(I) Therapy with retreat	38	0.36	0.40	*Ref*			
(II) Therapy without conspecifics	29	-0.01	0.41	-0.38	-0.52 to -0.23	< 0.001	***
(III) Therapy without retreat	36	0.20	0.39	-0.16	-0.28 to -0.04	0.012	*
(IV) Control	36	0.00	0.40	-0.36	-0.48 to -0.25	< 0.001	***

N, number of sessions; M, mean relative to baseline temperature in degree Celsius; SD, standard deviation; Coef, coefficient (effect size); CI, confidence interval.
* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ statistically significant compared to setting I (Therapy with retreat).

3.1.1 Temperature changes relative to baseline temperature within each setting

Eye temperature changes relative to the baseline temperature led to the following results: The therapy setting with retreat (I) and without retreat (III) resulted in an increase of the mean eye temperature of 0.36 ± 0.40 °C and 0.20 ± 0.39 °C, respectively, relative to the baseline temperature. No change in the mean eye temperature compared to baseline was found during therapy without conspecifics (II) and the control setting (IV).

3.1.2 Comparison of relative eye temperatures between settings

Comparing the relative eye temperatures between the different therapy settings led to the following results: In the therapy setting with retreat (I), there was a greater increase in mean eye temperature relative to baseline than in all other settings (II – IV) (Table 2, Figure 3).

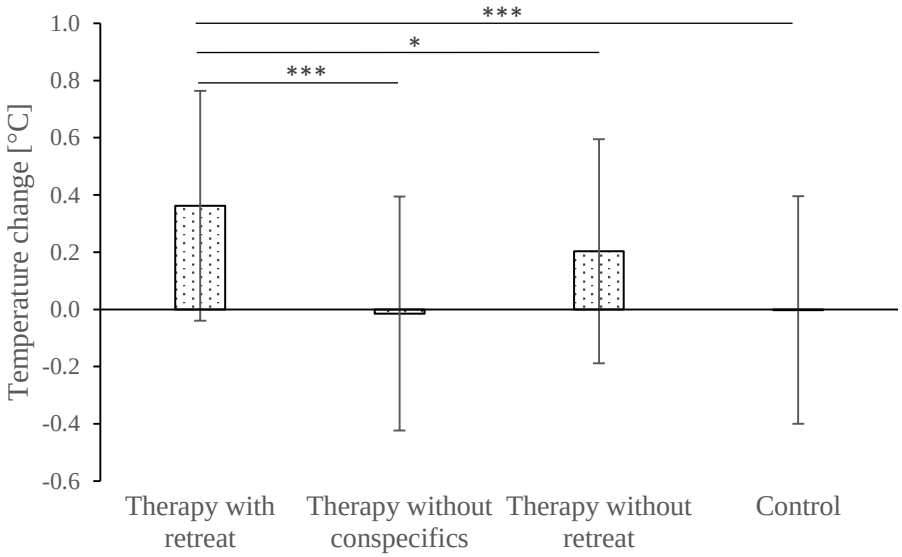

*Figure 3: Mean eye temperature changes relative to the baseline for the different settings. Error bars denote one standard deviation of the mean, * p < 0.05, *** p < 0.001 statistically significant compared to setting I (therapy with retreat).*

The day of data collection (one to six) had an influence on thermography values. As data collection for each guinea pig group progressed, the measured mean eye temperature dropped continuously from collection day one to collection day six (b = -0.05, CI = -0.09 to – 0.03 *P* = 0.035). For this reason, the models were also run with the data collection day as covariate. The results did not differ significantly from the results presented above and the significance levels of the *P*-values remained the same.

3.2 State behaviors compared between different settings

Table 3: State behaviors in percentage of the observed time compared between different settings.

	Behavior	**Setting**	**N**	**M**	**SD**	**Coef**	**95% CI**	***P* value**	
Location	Hiding	(I) Therapy with retreat	38	26.67	24.37	*Ref*			
		(II) Therapy without conspecifics	36	28.87	31.29	1.42	-6.11 to 8.95	0.71	
		(IV) Control	37	18.28	15.03	-6.00	-13.01 to 1.01	0.09	
	In enclosure unsheltered	(I) Therapy with retreat	38	37.56	25.46	*Ref*			
		(II) Therapy without conspecifics	36	55.27	32.39	11.84	3.84 to 19.83	0.004	**
		(IV) Control	37	59.47	21.37	15.16	8.27 to 22.05	< 0.001	***
	On board part	(I) Therapy with retreat	38	35.77	30.56	Ref			
		(II) Therapy without conspecifics	36	15.87	25.75	-19.30	-26.65 to -11.95	< 0.001	***
		(IV) Control	37	22.25	19.69	-9.19	-18.37 to -0.01	0.050	
Locomotion	Moving	(I) Therapy with retreat	38	12.26	4.96	*Ref*			
		(II) Therapy without conspecifics	36	10.74	6.63	-2.67	-6.18 to 0.84	0.14	
		(III) Therapy without retreat	36	7.34	7.48	-6.27	-9.46 to -3.08	< 0.001	***
		(IV) Control	37	5.79	2.59	-6.52	-8.36 to -4.68	< 0.001	***
	Standing Still	(I) Therapy with retreat	38	85.27	6.71	*Ref*			
		(II) Therapy without conspecifics	36	81.69	21.19	-2.26	-7.96 to 3.45	0.44	
		(III) Therapy without retreat	36	75.45	19.47	-5.44	-10.48 to -0.39	0.035	*
		(IV) Control	37	90.96	7.64	5.48	2.70 to 8.26	< 0.001	***

	Freezing	(I) Therapy with retreat	38	0.68	0.72	*Ref*			
		(II) Therapy without conspecifics	36	1.68	4.68	0.17	-1.63 to 1.97	0.85	
		(III) Therapy without retreat	36	17.17	17.79	17.59	12.11 to 23.07	< 0.001	***
		(IV) Control	37	1.51	2.40	1.71	0.52 to 2.89	0.005	**
Ingestion	Feeding time	(I) Therapy with retreat	38	78.10	10.76	*Ref*			
		(II) Therapy without conspecifics	35	69.73	30.49	-6.21	-15.10 to 2.68	0.17	
		(III) Therapy without retreat	36	51.33	31.45	-18.28	-26.76 to -9.80	< 0.001	***
		(IV) Control	37	87.22	9.13	7.15	3.56 to 10.74	< 0.001	***
Passive HAI	Petted	(I) Therapy with retreat	38	0.79	1.16	*Ref*			
		(II) Therapy without conspecifics	35	0.18	0.65	-2.65	-4.08 to -1.21	< 0.001	***
		(III) Therapy without retreat	36	28.22	20.53	26.93	21.60 to 32.26	< 0.001	***
	Held	(I) Therapy with retreat	38	0.00	0.00	*Ref*			
		(II) Therapy without conspecifics	35	0.00	0.00	0.00	0.00 to 0.00	-	
		(III) Therapy without retreat	36	7.56	8.68	14.20	11.26 to 17.13	< 0.001	***
Vocalization		(I) Therapy with retreat	38	0.97	1.45	*Ref*			
		(II) Therapy without conspecifics	36	0.32	0.73	-2.43	-4.25 to -0.61	0.009	**
		(III) Therapy without retreat	36	2.13	5.95	0.85	-2.76 to 4.45	0.65	
		(IV) Control	37	0.80	1.56	-0.28	-2.08 to 1.53	0.77	

HAI, human animal interaction; N, number of sessions; M, mean in % of observed time; SD, standard deviation; Coef, coefficient (effect size), CI, confidence interval.
* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ statistically significant compared to setting I (therapy with retreat).

Location

The percentage of time spent hidden in a shelter did not differ between the settings (Table 3). The guinea pigs more often came to the board part of the enclosure in the setting with retreat and conspecifics (I) compared to the therapy with retreat but without conspecifics (II) (b = -19.30, CI = -26.65 to -11.95, $P < 0.001$) and the control setting by trend (IV) (b = -9.19, CI = -18.37 to -0.01, $P = 0.050$). They spent more time in the enclosure part but outside of shelters in the therapy without conspecifics but with retreat (II) (b = 11.48, CI = 3.84 to 19.83, $P = 0.004$) and in the control setting (IV) (b = 15.16, CI = 8.27 to 22.05, $P < 0.001$) compared to therapy with conspecifics and retreat (I).

Locomotive Behavior

The guinea pigs spent more time moving around in the therapy with retreat (I) than in the therapy without retreat (III) (b = -6.27, CI = -9.46 to -3.08, $P < 0.001$) and the control setting (IV) (b = -6.52, CI = -8.36 to -4.68, $P < 0.001$) (Table 3). On the other hand they spent less time standing still in the therapy with retreat (I) compared to the control setting (IV) (b = 5.48, CI = 2.70 to 8.26, $P < 0.001$). In the therapy without retreat (III) the percentage of time spent freezing was much higher than in the therapy with retreat (I) (b = 17.59, CI = 12.11 to 23.07, $P < 0.001$).

Ingestion (Figure 4)

The guinea pigs spent the most time eating in the control setting (IV). Compared to that, time spent eating was significantly reduced in the therapy setting with retreat (I) (b = 7.15, CI = 3.56 to 10.74, $P < 0.001$). Moreover, the guinea pigs spent less time eating in the therapy setting without retreat (III) compared to the therapy with retreat (I) (b = -18.28, CI = -26.76 to – 9.80, $P < 0.001$). No significant differences in eating behavior were observed between the therapy with retreat (I) and the therapy without conspecifics (II).

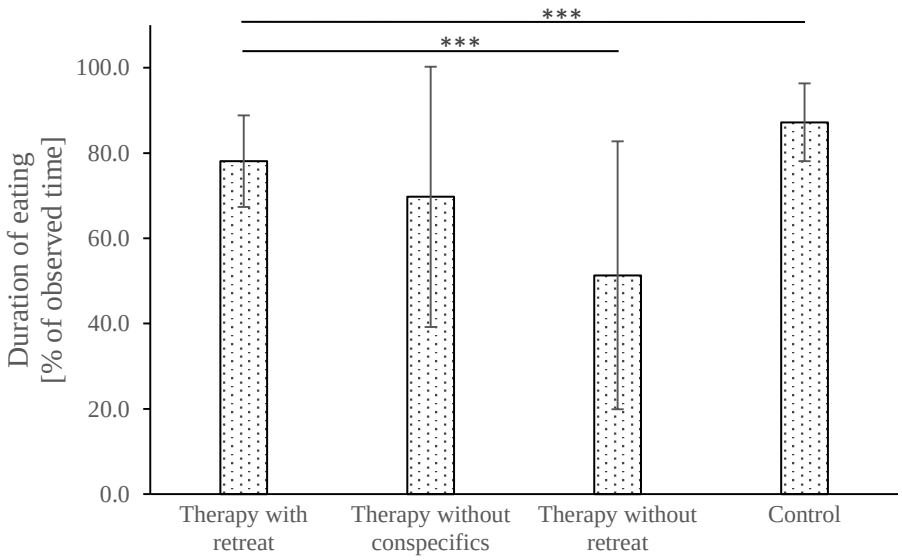

*Figure 4: Duration of eating during the different settings. Error bars denote one standard deviation of the mean, *** p < 0.001* statistically significant compared to setting I (therapy with retreat)

Passive HAI

The guinea pigs were petted (b = 26.93, CI = 21.60 to 32.26, $P < 0.001$) and held (b = 14.20, CI = 11.26 to 17.13, $P < 0.001$) longer during therapy without retreat (III) compared to therapy with retreat (I). They allowed being petted longer during therapy with conspecifics (I) compared to therapy without conspecifics (III) (b = -2.65, CI = -4.08 to -1.21, $P < 0.001$).

Vocalization

Guinea pigs spent less time vocalizing in the therapy without conspecifics (II) in comparison to the therapy with conspecifics (I) (b = -2.43, CI = -4.25 to -0.61, *P* = 0.009).

3.3 Count behaviors compared between different settings

Table 4: Frequencies count behaviors compared between different settings.

Behavior	Setting	N	M	SD	RR	95% CI	*P* value	
Hiding	(I) Therapy with retreat	38	0.28	0.21	*Ref*			
	(II) Therapy without conspecifics	36	0.30	0.26	1.06	0.75 to 1.50	0.73	
	(IV) Control	37	0.15	0.11	0.53	0.40 to 0.69	< 0.001	***
Startling	(I) Therapy with retreat	38	0.16	0.17	*Ref*			
	(II) Therapy without conspecifics	36	0.10	0.23	0.56	0.26 to 1.18	0.13	
	(III) Therapy without retreat	36	0.03	0.13	0.25	0.07 to 0.87	0.03	*
	(IV) Control	37	0.03	0.05	0.25	0.14 to 0.44	< 0.001	***
Freezing	(I) Therapy with retreat	38	0.14	0.14	*Ref*			
	(II) Therapy without conspecifics	36	0.23	0.40	1.74	0.99 to 3.08	0.06	
	(III) Therapy without retreat	36	1.41	0.96	12.02	8.40 to 17.20	< 0.001	***
	(IV) Control	37	0.15	0.12	1.22	0.94 to 1.57	0.13	
Explorative behavior	(I) Therapy with retreat	38	0.03	0.06	*Ref*			
	(II) Therapy without conspecifics	36	0.03	0.16	0.79	0.23 to 2.64	0.70	
	(III) Therapy without retreat	36	0.01	0.03	0.23	0.03 to 1.60	0.14	
	(IV) Control	37	0.01	0.03	0.41	0.16 to 1.07	0.07	
Comfort behavior	(I) Therapy with retreat	38	0.14	0.09	*Ref*			
	(II) Therapy without conspecifics	36	0.07	0.16	0.48	0.22 to 1.04	0.06	
	(III) Therapy without retreat	36	0.07	0.19	0.76	0.30 to 1.94	0.57	
	(IV) Control	37	0.12	0.10	0.97	0.70 to 1.35	0.86	
Socionegative active HAI	(I) Therapy with retreat	38	0.06	0.07	*Ref*			
	(II) Therapy without conspecifics	36	0.02	0.09	0.21	0.06 to 1.79	0.02	*
	(III) Therapy without retreat	36	0.41	0.68	6.98	3.96 to 12.30	< 0.001	***

HAI, human animal interaction; N, number of sessions; M, mean per 60 seconds; SD, standard deviation; RR, rate ratio (effect size); CI, confidence interval.
* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ statistically significant compared to setting I (therapy with retreat).

Hiding

Guinea pigs retreated more frequently into shelters in the therapy with retreat (I) (b = 0.53, CI = 0.40 to 0.69, $P < 0.001$) compared to the control setting (IV) (Table 4).

Startling

The guinea pigs startled more often in the setting with retreat (I) compared to the control setting (IV) (b = 0.25, CI = 0.14 to 0.44, $P < 0.001$) and compared to the setting without retreat (III) (b = 0.25, CI = 0.07 to 0.87, $P = 0.03$) (Figure 5).

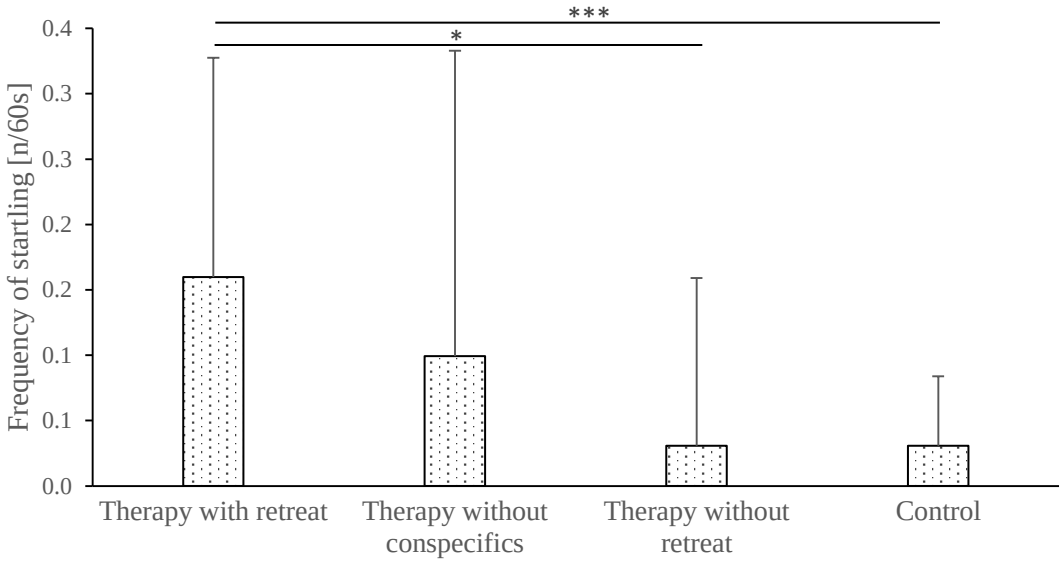

*Figure 5: Frequency of startling behavior in the different conditions. Error bars denote one standard deviation of the mean, * p < 0.05, *** p < 0.001 statistically significant compared to setting I (therapy with retreat).*

Freezing

Freezing occurred more often when guinea pigs were on the lap and thus had no retreat available (III) (b = 12.02, CI = 8.40 to 17.20, $P < 0.001$) compared to the therapy with retreat (I) (Figure 6).

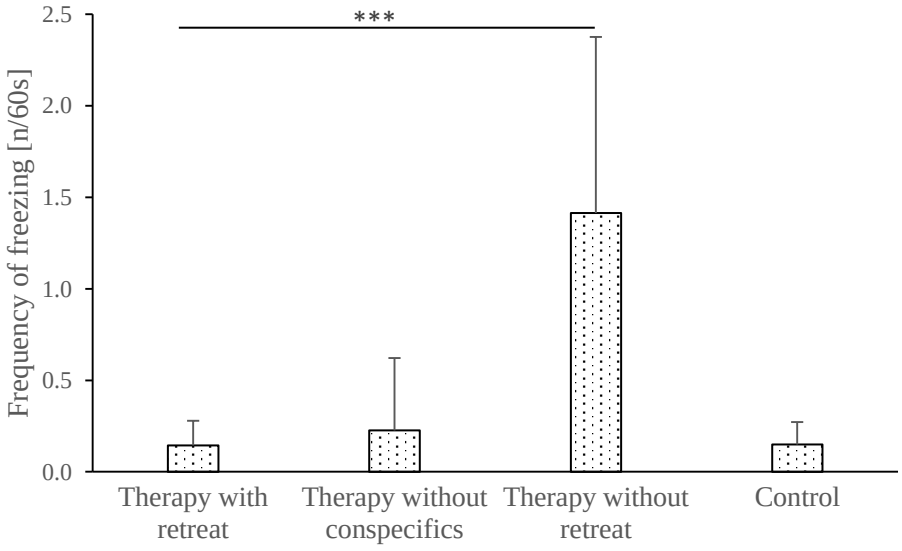

*Figure 6: Frequency of freezing behavior in the different conditions. Error bars denote one standard deviation of the mean, *** p < 0.001 statistically significant compared to setting I (therapy with retreat).*

Explorative and comfort behavior

No significant differences in frequencies of explorative or comfort behaviors were found between the settings I to IV. There was a tendency that explorative behavior was shown more often in therapy with retreat (I) compared to the control setting (IV) (b = 0.41, CI = 0.16 – 1.07, *P* = 0.07) (Table 4).

Socionegative active HAI

Guinea pigs showed more socionegative interactions towards humans in the therapy without retreat (III) (b = 6.98, CI = 3.69 to 12.30, *P* < 0.001) compared to therapy with retreat (I). They also showed more socionegative interactions towards humans in the setting with conspecifics (I) compared to the setting without conspecifics (II) (b = 0.21, CI = 0.06 to 0.79, *P* = 0.02).

3.4 Other factors

The longer the guinea pigs were petted during the settings, the higher the increase in eye temperature was relative to baseline (b = 0.57, CI = 0.14 to 1.00, *P* = 0.010). The day of data collection had a significant influence on eye temperature during setting and was, therefore, used in the models (see section 3.1). The following factors did not have a significant influence on thermography results: sex, absolute room temperature and experience in AAT prior to this study (see Appendix, Table 6).

The absolute room temperature had an effect on behavior. Higher room temperatures at the beginning of each data collection correlated with more frequent hiding (b = 1.17, CI = 1.09 to 1.26, *P* < 0.001) and less time on the board part with the pet bed (b = - 3.33, CI = - 5.90 to - 0.76, *P* = 0.011). Higher room temperatures correlated with longer time spent freezing (b = 0.88, CI = 0.02 to 1.73, *P* = 0.44) as well as the tendency for more frequent freezing (b = 1.12, CI = 0.99 to 1.27, *P* = 0.074). The animals spent less time eating (b = - 2.04, CI = - 4.03 to -

0.055, *P* = 0.044) and less time vocalizing (b = - 0.71, CI = - 1.24 to - 0.17, *P* = 0.01) at higher temperatures.

The guinea pigs who already had experience in AAT prior to the study hid (b = 0.59, CI = 0.39 to 0.90, *P* = 0.013), startled (b = 0.28, CI = 0.13 to 0.59, *P* < 0.001) and froze (b = 0.40, CI = 0.26 to 0.59, *P* < 0.001) less frequently than animals who had not been previously involved in AAT. Furthermore "AAT-experienced" guinea pigs spent more time standing still (b = 7.84, CI = 4.15 to 11.53, *P* < 0.001) and eating (b = 10.71, CI = 3.70 to 17.72, *P* = 0.003) than the guinea pigs who had no prior experience with AAT.

Female guinea pigs were petted longer than male guinea pigs (b = 3.31, CI = 0.20 to 6.42, *P* = 0.037).

Day of data collection did not have a significant influence on behavioral results (see Appendix, Tables 7 and 8).

4. Discussion

We identified the availability of a retreat, the presence of conspecifics and human contact as important factors affecting welfare in guinea pigs involved in AAT. The influence of these three factors are considered consecutively.

Availability of a retreat and its influence on welfare of guinea pigs

According to our hypothesis that provision of a retreat and giving the animal free choice of interactions are associated with reduced physiological and behavioral stress indicators, we expected a higher physiological stress level and therefore a larger rise in eye temperature in the setting without retreat (III) compared to the setting with retreat (I). However, we could not confirm this hypothesis. During both settings an increase in eye temperature was measured, and the increase in the setting with retreat (I) was even statistically significantly higher than in the setting without retreat (III).

In contrast to this, the behavior results indicate that the availability of a retreat has a significant effect on the welfare of the guinea pigs. In the setting without retreat possibility, the guinea pigs spent significantly less time eating compared to the setting with retreat possibility. Also, the guinea pigs showed more active behaviors (e.g. more movement, more frequent startling) when they had the possibility to retreat, whereas they showed more passive behaviors (e.g. more standing still, longer and more frequent freezing) with no possibility to retreat. This indicates that a possible enriching effect of human contact is only there when the guinea pigs have retreat possibilities. Guinea pigs showed more frequent socionegative human-animal interactions (e.g. head-up and sudden locomotion away from the human) without retreat possibility. All these behavioral results are in line with our hypothesis and with our previous study [15], leading to the conclusion that the lack of retreat can lead to more stress and thus a reduced welfare in guinea pigs.

The physiological and behavioral results differ. While according to behavioral data the guinea pigs seem to show more stress-associated behaviors when no retreat is available, the physiological arousal is even higher when retreat is possible. This poses a challenge in interpreting our results. However, it must be considered that the measured physiological arousal might also be caused by positive excitement or physiological exercise [32,33]. It is possible that the higher active coping in the therapy setting with retreat possibility versus the more passive coping in the therapy setting without retreat possibility might be correlated with a higher physical arousal.

Presence of conspecifics and its influence on welfare of guinea pigs

We hypothesized that the presence of conspecifics leads to reduced physiological and behavioral stress indicators during AAT. To test this hypothesis, we compared the therapy setting with retreat with access to conspecifics (I) to the therapy setting with retreat without access to conspecifics (II). Contrary to our hypothesis, we found that guinea pigs had a significantly higher eye temperature in the presence of conspecifics compared to their absence. Regarding behavioral changes, we found that the guinea pigs came less often to the board part and spent more time in the table enclosure when conspecifics were absent. Previous research showed that the presence of bonding partners could reduce physiological and behavioral reactions in guinea pigs in stressful situations [8,34]. Sachser and colleagues [8] call this phenomenon ‘security-giving and arousal-reducing structure’. Our findings only partly support this, since we measured higher physiological arousal in the presence of conspecifics. But as noted, whether the increased eye temperature was caused by negative stress, increased physical activity, positive arousal or a combination of different factors remains unclear and requires further research.

Interestingly, the guinea pigs in our study vocalized less often when alone compared to being with their social group. This result indicates that vocalizing in our study was mainly

communication between conspecifics in the same enclosure rather than calling for conspecifics when isolated or communication with the patient.

Human-animal interaction and its influence on welfare of guinea pigs

Based on our previous study [15], we hypothesized that human-animal interaction could be an enrichment for the guinea pigs leading to interaction with the human and with the environment. To test this hypothesis, we compared the therapy setting with retreat (I) with human interaction to the control setting (IV) without human interaction.

The presence of a human, and thus human-animal interaction, led to an increase of the eye temperature compared to the control setting, indicating a higher physiological arousal assumedly due to human contact. This can be interpreted as higher negative stress or might reflect a higher positive excitement. An increase in short-term changes in body temperature can be due to stressful situation [20,22,23,26–28]. However, it can also be driven by other factors such as physiological exercise [32] or a positive emotional state [33] (e.g. due to a particularly tasty food offer for guinea pigs). Whether the increased body temperature in the setting with human-animal interaction is caused by negative stress, increased physical activity, positive arousal or a combination of different factors remains unclear and requires further research. Still, we found a significant relationship between the length of time a guinea pig was petted and the rise of the eye temperature independent of the setting. These findings indicate that petting might lead to increased stress in guinea pigs. Guinea pigs are highly social animals living in a group with strong social bonds. Nevertheless, they show very little close body contact with their conspecifics [35,36]. This could be a reason why physical contact from humans, such as petting, might cause stress in guinea pigs.

Behavior results revealed that guinea pigs in the control setting spent most of their time in the table enclosure standing still (but not freezing), whereas they showed significantly more locomotive behavior and came to the board part much more frequently in the therapy condition

with retreat, even though the same amount and type of food was available in both conditions. Moreover, there was a tendency for guinea pigs to show more explorative behavior when a human was present. These results indicate that human contact might indeed have an enriching effect on guinea pigs. However, we also found decreased time spent eating, increased frequency of hiding and increased startling in the guinea pigs exposed to human contact. These results might indicate that the presence of a human can increase stress-levels in guinea pigs involved in AAT which can lead to a reduced welfare. We interpret that human contact might lead to an enrichment, but at the same time induce a certain amount of stress. However, the stress is not considered excessive because, although they were hiding more frequently, they did not hide in a shelter for a longer period in total. Moreover, no extreme stress-associated behaviors (e.g., eye-closing, piloerection, or attempts to flee like jumping out of the pet bed on the patient's lap) were observed in any therapy setting, and no session had to be stopped due to break-off criteria.

Influence of other factors

The guinea pigs' prior experience in AAT did not have an influence on eye temperature. However, AAT-experienced guinea pigs showed significantly less stress-associated behaviors such as hiding, startling and freezing compared to inexperienced animals. This difference supports the assumption that guinea pigs adapt to certain situations over time and are habituated to being involved in AAT. This is in line with literature of Miller et al. [37] and also seen in other species [38]. Moreover, we found a reduction of the increase in eye temperature over the course of the test days. We interpret this result to be associated with habituation to the situation over the repeated sessions. Contrary to this, we found no indicators for habitation over time based on behavioral observations. It is therefore possible that physiological arousal is more sensitive to habituation than is behavior. We did not find an influence of sex on either eye temperature or behavioral outcomes. This is not surprising since we controlled for influences

of the sexual cycle of intact females and the differences between sexes by analyzing changes in the eye temperature relative to a baseline. Moreover, we only included intact females and spayed males, which should not display typical male sexual-associated behaviors.

Limitations

The behavioral coding in this study could not be blinded. However, as in the previous study [15], the person coding the videos was not involved with AAT before the study and we used the same detailed coding scheme. Moreover, intra-rater and inter-rater reliability was high. The problem of multiple testing was carefully examined [39]. Since it was impossible to avoid multiple testing completely, the focus was set on reducing the number of specific questions. However, this study must be viewed as explorative, and results must be interpreted with caution. Another limitation is that it remains unclear which factor such as negative stress, physical activity or positive state, or which combination of factors led to the increased eye temperature which itself only reflects physiological arousal [22,23,32,33]. On the basis of our results, it is not possible to say if or to what extent an increase of the eye temperature is an indicator for increased stress in guinea pigs involved in AAT. Moreover, the low number of investigated animals and the relatively high variability of the values led to a relatively large standard deviation in the physiology results. Therefore, physiological data should always be interpreted together with behavioral data and in the context of circumstances that might help to explain the valence of arousal. On the other hand, observed behavior must also be interpreted with caution. Guinea pigs have very complex communication and behavior patterns [8,36]. In this study, the clear and distinct behaviors were coded with the ethogram (see Appendix, Table 5). However, it is possible that there are much more subtle behaviors, which are also associated with stress, that were not perceptible in the video recordings. On the other hand, it is unclear to what extent our interpretation of the guinea pig observed behaviors correspond with their perception, health or longevity outcome, although we do have a simultaneous physiological measurement. As a

prey species, they are likely adapted to frequent, but short, stressful situations. We did not measure the duration of the stress levels due to AAT. Our results indicate that they can learn to cope with stressful situations due to the fact that experienced guinea pigs showed fewer stress behaviors. Further research on the long-term implications on guinea pig health and longevity should be done in the future. We interpret an increase in locomotion and explorative behavior as enrichment according to Brewer et al. [40]. Other specific behaviors such as spending time on the board part might also be signs of enrichment. Moreover, we assume that these behaviors are directly associated with welfare [41–43] and that welfare is enhanced with more enrichment [41]. Negative stress, in contrary, is assumed to reduce welfare [2]. However, these are interpretations that need further investigations.

Strengths

This is the first study investigating physiological as well as behavioral effects in guinea pigs assisting in AAT. We replicated findings of our previous study [15] in a larger population of guinea pigs and extended the design. In this study, the rise in eye temperature as an estimate of core body temperature served as an indicator of physiological stress. Previously, physiological stress in guinea pigs has been studied using saliva cortisol levels or fecal glucocorticoid metabolite concentrations [44]. To our knowledge, this is the first study to use eye temperature of guinea pigs to assess stress levels. Moreover, we investigated the effect of different factors, human contact, retreat possibility, and presence of conspecifics, by comparing different types of settings. The randomized assignment of the order of the different settings for each individual guinea pig ensured that habituation effects did not influence the results of the study. We also carefully controlled for confounding factors to ensure high internal validity. To reduce variance and to standardize interactions as much as possible, we did not work with real patients. This ensured that the guinea pigs were handled in the same way in each setting. However, we designed the interactions in a very similar way to real situations to reflect actual practice. The

person acting as patient observed several actual therapy sessions before the study start. This ensures high external validity.

Implications for research

This study is a next step toward understanding the effects of human-animal interaction on guinea pig well-being in general, and specifically the effects of integrating guinea pigs into AAT. Although we could include previously suggested methodological improvements, such as a higher number of included animals, combining behavioral and physiological measurements and testing for social buffering, many open questions still remain. Future research should investigate the effect of personality of the guinea pigs as well as their extent of experience in AAT and experience in contact with humans in general on welfare. Another important factor would be the influence of the bond the animals have with the present human handler. In our study, the person interacting with the guinea pigs was an unknown person when the study started. In future research, the effect of humans with whom they have a social bond interacting with the guinea pig should be compared to that of unfamiliar humans.

Future studies should try to disentangle the possible causes of physiological arousal by varying the intensity of stressors, physiological exercise and the positive excitement due to e.g. a bonding partner, food or a cue that indicates food. Moreover, the meaning and clinical relevance of the observed physiological and behavioral changes need further investigation. It still remains unclear what amount of change is considered as “distress” or “eustress” and what intensity may be associated with health problems.

Implications for practice

Our study indicates that – given the right conditions – involving guinea pigs does not cause excessive stress and could potentially even act as an enrichment. Guinea pigs can be suitable animals for animal-assisted interventions [4–7] but need species-appropriate handling and

housing. Our results are not only relevant for human interaction with guinea pigs in AAT but also for general housing and handling of guinea pigs. In alignment with our previous study [15] and other research [1,45], it can be concluded that guinea pig welfare can be improved by giving them the possibility to interact with their environment. They should have freedom to choose to interact with humans or retreat to a hiding place when they feel insecure or afraid. If guinea pigs have control over the situation, they will gain more security, which leads to improved well-being and reduced stress. Moreover, guinea pigs should only be petted if the animals itself can determine how long and intense the contact is and if they can withdraw when they choose. It is also important that the guinea pigs have time to adapt to a new setting and stimuli and that they are used to human contact. Guinea pigs with less experience in AAT showed higher stress-associated behaviors than those with more experience.

Since each individual reacts quite differently to variable stimuli, it is not possible to draw general rules on how to work with guinea pigs in AATs from the existing research, apart from these conclusions. However, we highly recommend including these conclusions into best-practice guidelines, until there is new research. Moreover, it is the responsibility of the animal owners to ensure that they know each individual very well. This helps to increase the validity of the interpretation of the reactions of a specific guinea pig. For owners and people working with guinea pigs in AAT, guinea pig behaviors are the only source to identify welfare of their animals. It is therefore crucial to recognize behaviors that are indicators of welfare. Existing literature has already identified behaviors that can be regarded as stress indicators in guinea pigs [46,47], including decreases in eating, decreased exploration and fewer social behavior. In line with this, we also identified a reduced time spent eating as possibly stress-associated. Additionally, we identified two more behaviors that can be indicators of increasing stress level: an increased frequency of startling and an increased frequency and duration of freezing.

5. Conclusions

Our results suggest that human contact, availability of retreat and presence of conspecifics have important influences on the welfare in guinea pigs involved in AAT. Petting was identified as a key factor leading to stress in guinea pigs because it was associated with an increase in eye temperature. Based on our results, we identified the behaviors “reduced eating”, “increased startling” and “increased freezing” as indicators of increasing stress level and reduced welfare. This study supports our previous hypotheses that a “good practice” for guinea pig-assisted interventions includes retreat possibilities for the animals whenever possible to ensure free choice of human interaction and presence of conspecifics. Moreover, it is important that the involved animals have time to adapt and are carefully accustomed to new settings. In this way, stress can be reduced, and AAT might potentially even constitute enrichment for the involved animals. Considering our observations, we can plan AAT in a way to minimize the tradeoff of human health benefits against reduced animal welfare, within the paradigm of One Health, seeking incremental benefits from a closer collaboration of human and animal health.

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Appendix

Ethogram (according to Gut and colleagues [15]):

Table 5: The observed behaviors were grouped in the following categories:

Behavioural Categories	*Behavioural Groups*	*Observed behaviours*	*Subbehaviours*	*Definitions*
Individual Behaviour	**Ingestive Behaviour**	Eating with human interaction	-	Consuming food which is acquired through human interaction.
		Eating without human interaction	-	Consuming food which is not acquired through patient interaction.
		Not eating	-	Not consuming food.
	Locomotive Behaviour	Locomotion	-	Moving from one place to another in cage; 2 or more paws take steps.
		Resting	-	Laying stomach down on ground while relaxing or sleeping.
		Standing still	-	Standing on at least 3 feet while staying in one spot. Movements with other parts such as head ongoing.
		Freezing	-	Freezing in place; cessation of all movements for a timespan > 1s.
		Jumping	-	Sudden locomotion directed mostly upwards, not caused by a trigger.
		Startling	-	Sudden and jerky movement directed mostly upwards due to a trigger.
	Comfort Behaviour	Comfort Behaviour	*Sneezing*	Sudden and involuntary burst of air pressed from lungs through nose at a high velocity.
			Coughing	Burst of air pressed from lungs through throat and mouth.

			Yawning	Deep inhalation of air combined with wide opened mouth.
			Shaking	Rapid side to side movement of body or part of body (example head).
			Stretching	Straightening or extending body or part of body.
Animal-Environment Interaction	**Explorative behaviour**	Explorative behaviour	*Gnawing objects*	Gnawing on objects in cage using teeth without eating.
			Pushing objects around	Displacing objects by pushing them with nose.
			Digging	Displacing litter with several strokes of the front and back limbs.
	Location in Table Cage	Hiding	-	Hiding so that at least 50% of animal is covered by shelter.
		On table	-	Located on table part of the cage.
		In cage unsheltered	-	Located within boundaries of the cage, but not hiding within a shelter or on table part of cage.
Social behaviour	**Sociopositive behaviour**	Sociopositive behaviour	*Nose-nose*	Two encountering animals touch noses and sniff each other.
			Body sniff	Sniffing a part of another animals' body.
	General socionegative behaviour	socionegative defensive behaviour	*Retreating*	Sudden locomotion away from another animal with an increase in distance of more than one body length.
			Head-up	Head is thrown back and nose is pointed straight upwards.
			Kicking	Using one or both hind feet, directed toward another animal
			Head-thrust	Thrusting of head towards another animal.

			Stand-threat	Curved body posture is displayed by two animals toward each other.
		Socionegative offensive	*Attack-lunge*	Short run or jump towards another animal.
			Chasing	Pursuing an animal which is moving away.
			Facing	Turning around to face an animal at the rear in a sudden motion.
			Pawing	Foot motions same as in digging but with faster strokes.
Animal Human Interaction	**sociopositive active AHI**	sociopositive active AHI	*Sniffing*	Repeatedly drawing air up nose to detect a smell, directed at a human or a part of a human.
			Licking	Rhythmic movements of tongue on a person's skin.
			Body contact	Contact with a human initiated specifically by guinea pig, without any other specific sociopositive or socionegative behaviour.
	General socionegative active AHI	Socionegative defensive active AHI	*Retreating*	Sudden locomotion away from human with an increase in distance to the human of more than one body length.
			Head-up	Head is thrown back and nose is pointed straight upwards.
		Socionegative offensive active AHI	*Kicking*	Using one hind foot or both hind feet, directed at hand of a human at rear.
			Head-thrust	Thrusting of head towards human.
			Biting	Closing of teeth on skin of human.
	Other AHI	Eating with patient interaction	-	Consuming food which is acquired through patient interaction.
	Passive AHI	**Being stroked**	-	Guinea pig is stroked by human.

Vocalisation	**Vocalisation**	Vocalisation	*Bubbly squeak*	Mixed variety of notes.
			Whistle	High pitched whistle.
			Squeal	A scream-like, high pitched note.
			Chirps	High pitched, rapidly repeated notes of same frequency.
			Grunt	Multiple low, throaty notes.
			Rumble	Series of low, pitched notes similar to purring.
			Tooth chatter	Noise made by rubbing teeth together, made visible by jaw movements.

Influence of additional factors

Table 6: Influence of additional factors on thermography results

Physiology	Other factors	Coef	95% CI		*P* value	
Relative eye temperature	Sex	0.15	- 0.12	0.43	0.27	
	Day of data collection	- 0.05	- 0.09	- 0.03	0.035	*
	Experience in AAT	0.081	- 0.10	0.26	0.38	
	Absolute room temperature	- 0.012	- 0.072	0.047	0.69	

Coef, coefficient (effect size); CI, confidence interval.
* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ statistically significant

Table 7: Influence of additional factors on state behaviors

Behavior	Other factors	Coef	95% CI		*P* value	
Hiding	Sex	3.42	- 10.22	17.06	0.62	
	Day of data collection	- 0.95	- 3.14	1.24	0.39	
	Experience in AAT	- 2.11	- 12.72	8.50	0.70	
	Absolute room temperature	1.25	- 1.35	3.85	0.35	
In enclosure unsheltered	Sex	- 1. 99	- 13.52	9.54	0.74	
	Day of data collection	0.63	- 1.42	2.69	0.55	
	Experience in AAT	- 9.26	- 20.08	1.56	0.093	
	Absolute room temperature	1.90	- 0.43	4-23	0.11	
On board part	Sex	- 2.73	- 15.01	9.54	0.66	
	Day of data collection	0.46	- 1.54	2.45	0.65	
	Experience in AAT	12.19	- 2.10	26.48	0.094	
	Absolute room temperature	- 3.33	- 5.90	- 0.76	0.011	*
Moving	Sex	- 0.73	- 3.41	1.96	0.60	
	Day of data collection	0.45	- 1.14	0.24	0.20	
	Experience in AAT	- 1.74	- 3.98	0.50	0.13	
	Absolute room temperature	- 0.28	- 0.92	0.35	0.38	
Standing still	Sex	2.75	- 1.65	7.14	0.22	
	Day of data collection	0.34	- 1.26	0.17	0.68	
	Experience in AAT	7.84	4.15	11.53	< 0.001	***
	Absolute room temperature	- 0.61	- 2.07	0.86	0.42	
Freezing	Sex	0.93	- 2.48	4.34	0.59	
	Day of data collection	0.10	- 0.60	0.80	0.78	
	Experience in AAT	- 5.68	- 8,47	- 2.89	< 0.001	***
	Absolute room temperature	0.88	0.02	1.73	0.044	*
Feeding time	Sex	0.70	- 9.80	11.20	0.90	
	Day of data collection	- 0.28	- 2.03	1.48	0.76	
	Experience in AAT	10.71	3.70	17.71	0.003	**
	Absolute room temperature	- 2.04	- 4.03	- 0.055	0.044	*
Petted	Sex	3.31	0.20	6.42	0.037	*
	Day of data collection	0.022	- 0.80	0.84	0.96	

	Experience in AAT	1.80	- 1.81	5.42	0.33	
	Absolute room temperature	0.23	- 0.85	1.21	0.68	
Held	Sex	- 0.091	- 1.47	1.29	0.90	
	Day of data collection	- 0.29	- 0.84	0.26	0.30	
	Experience in AAT	- 2.30	- 3.95	- 0.66	0.006	**
	Absolute room temperature	- 0.009	- 0.55	0.53	0.97	
Vocalization	Sex	- 1.67	- 4.03	0.68	0.16	
	Day of data collection	0.18	- 0.027	0.62	0.44	
	Experience in AAT	1.70	- 0.94	4.35	0.21	
	Absolute room temperature	- 0.71	- 1.24	- 0.17	0.010	*

Coef, coefficient (effect size); CI, confidence interval.
* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ statistically significant

Table 8: Influence of additional factors on count behaviors

Behavior	**Other factors**	**RR**	**95% CI**		***P* value**	
Hiding	Sex	0.91	0.63	1.30	0.59	
	Day of data collection	1.02	0.93	1.12	0.72	
	Experience in AAT	0.59	0.39	0.90	0.013	*
	Absolute room temperature	1.17	1.09	1.26	< 0.001	***
Startling	Sex	0.74	0.39	1.41	0.36	
	Day of data collection	0.97	0.87	1.08	0.56	
	Experience in AAT	0.28	0.13	0.59	< 0.001	***
	Absolute room temperature	1.17	0.99	1.37	0.063	
Freezing	Sex	0.79	0.47	1.32	0.36	
	Day of data collection	0.97	0.90	1.05	0.47	
	Experience in AAT	0.40	0.26	0.59	< 0.001	***
	Absolute room temperature	1.12	0.99	1.27	0.074	
Explorative behavior	Sex	0.55	0.20	1.51	0.25	
	Day of data collection	0.91	0.72	1.15	0.43	
	Experience in AAT	1.66	0.60	4.59	0.33	
	Absolute room temperature	1.04	0.80	1.35	0.79	
Comfort behavior	Sex	0.93	0.64	1.36	0.72	
	Day of data collection	0.96	0.89	1.03	0.24	
	Experience in AAT	0.73	0.58	0.92	0.007	**
	Absolute room temperature	0.95	0.88	1.03	0.25	
Socionegative active HAI	Sex	1.16	0.46	2.97	0.75	
	Day of data collection	0.89	0.77	1.03	0.12	
	Experience in AAT	0.29	0.14	0.59	< 0.001	***
	Absolute room temperature	1.05	0.87	1.27	0.61	

HAI Human animal interaction; RR, rate ratio (effect size); CI, confidence interval.
* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ statistically significant

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